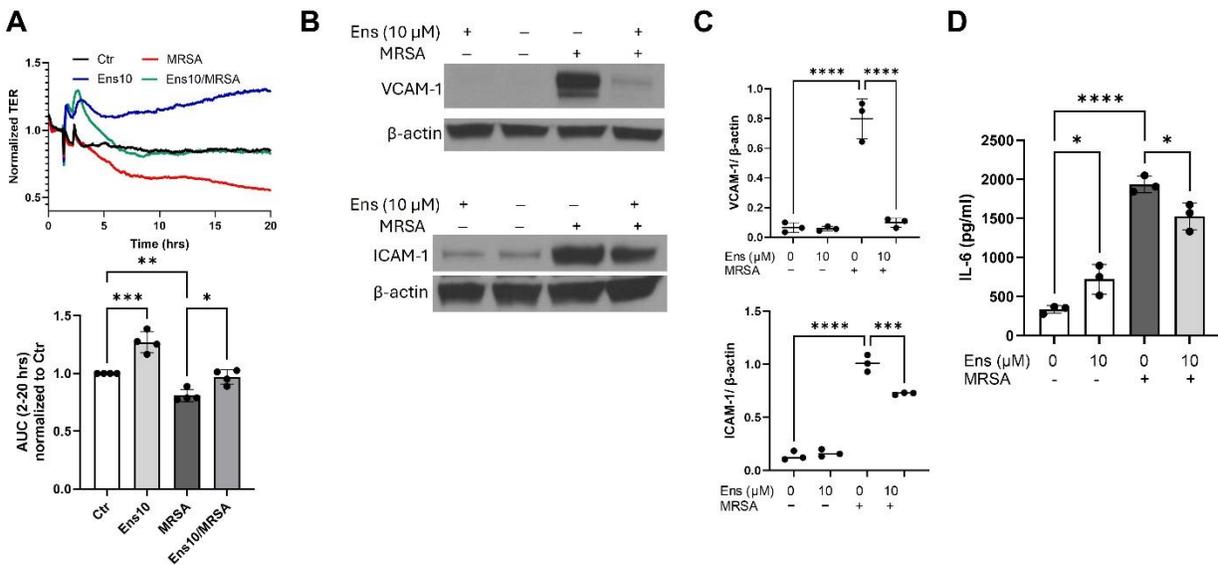
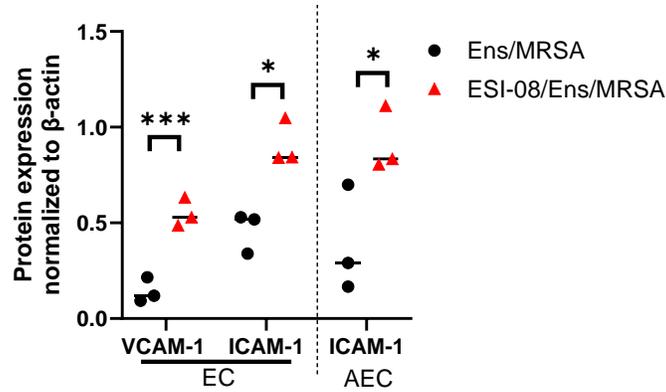


**Supplementary Figure 1. Early phase effects of ensifentrine on EC barrier function.** Human pulmonary artery endothelial cells (HPAEC) were treated with ensifentrine (5  $\mu\text{M}$ ) or vehicle (DMSO). EC barrier function was assessed using the ECIS assay. Depicted is a representative plot of normalized TER values recorded for 1 hour after addition of ensifentrine.



**Supplementary Figure 2. Effects of ensifentrine (10  $\mu\text{M}$ ) on lung EC barrier function after MRSA treatment.** HPAEC were pre-treated with ensifentrine (10  $\mu\text{M}$ ) or vehicle (DMSO) for 1 hour prior to HK-MRSA challenge ( $2.5 \times 10^8/\text{ml}$ ). EC barrier was assessed with the ECIS assay. **(A)** Representative TER tracings over time. Data were quantified after calculating the area under the curve for each condition. **(B-C)** Representative western blots of EC lysates for VCAM-1 and ICAM-1 expression and corresponding densitometry. **(D)** IL-6 levels were measured in EC

supernatants. N=3-4 independent experiments. Data were analyzed using one-way Anova, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001.



**Supplementary Figure 3. EPAC antagonism by ESI-08 alters the effects of ensifentrine on adhesion molecule expression in lung EC and AEC exposed to MRSA.** HPAEC and A549 were pre-treated with 20  $\mu$ M or 5  $\mu$ M ESI-08 (EPAC antagonist) respectively. After 1 hour, ensifentrine was added (5  $\mu$ M for HPAEC and 15  $\mu$ M for A549). Cells were treated with HK-MRSA 1 hour later ( $2.5 \times 10^8$ /ml, 20 hrs), and cell lysates were analyzed for VCAM-1 and ICAM-1 expression. Depicted is the densitometry analysis. Data were analyzed using t-test, \*p<0.05, \*\*\*p<0.001.